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An Experimental Investigation of Azalea Host Choice by the Leaf Beetle *Pyrrhalta rufosanguinea* (Coleoptera: Chrysomelidae)

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Rhododendron periclymenoides (Michaux) Shinners (=*R. nudiflorum* [L.] Torrey) and *R. atlanticum* (Ashe) Rehder are native azaleas and belong to the section *Pentanthera*. Fourteen of the 15 North American species of this section have overlapping ranges in the eastern United States. The species of the section have been classified into species groups called alliances or complexes based on systematic assessments of morphological and cytological characters (Skinner & Camp, 1952; Li, 1957) or flavonoid chemistry (King, 1980). All of these studies treated *R. periclymenoides*, *R. roseum* (Loisel.) Rehder, and *R. canescens* (Michaux) Sweet as an alliance. *Rhododendron atlanticum* has been variously placed in other alliances depending on whether morphological or chemical data were emphasized, although it has been reported (Galle, 1967) to hybridize with *R. periclymenoides* wherever their ranges overlap.

A field survey of *R. periclymenoides*, and co-occurring ericaceous shrubs for *Pyrrhalta rufosanguinea* (Say), suggested that this leaf beetle is monophagous for *R. periclymenoides* (King, 1993). Field observations suggested that, in mixed populations, *P. rufosanguinea* is restricted to *R. periclymenoides* or its introgressants (King, 1993).

The first objective of this experimental study was to test the hypothesis that, when *R. periclymenoides* and *R. atlanticum* co-occur, the former is the preferred host for *P. rufosanguinea*. The second objective was to test the hypothesis that these leaf beetles will use *R. atlanticum* as an alternate host when it is the only *Rhododendron* species available.

Materials and Methods

A colony of leaf beetles was maintained on freshly collected *Rhododendron periclymenoides* foliage in an environmental chamber. Plants for the experiments were sampled from three populations of *R. periclymenoides* in Caroline and Hanover counties, VA (*King 2220, 2230, 2310*) and from one population *R. atlanticum* in Henrico County, VA (*King 2240*). The locations of sites for beetle collections and methods for beetle colony maintainence are found in King (1993).

Leaf samples of each *Rhododendron* species were collected for feeding tests in the morning of the day each test was to begin. Although the leaf arrangement is alternate, leaves that develop from winter buds form a compact cluster at the end of a stem. A random foliage sample was 2-3 branches, each with several leaf clusters. The samples were sealed in plastic bags as they were removed from the plant, placed on ice in a cooler, and taken to the laboratory (*ca* 1 h). Only undamaged leaves from the foliage samples were used in the experiments. All feeding experiments were conducted in a growth chamber at 20° C with a 14 h photoperiod (400 foot candles) and 80-90% relative humidity. The duration of each feeding test was 24 h. The leaf beetles used in the feeding tests were taken at random from the laboratory maintained colony. No attempt was made to sort male and female beetles. The experimental design was modified from Villani & Gould (1985).

A paired choice feeding experiment was done in 10 tests from June 13 through June 22 with two tests each day. Feeding cages or arenas were one pint translucent plastic food containers with air holes. Plastic petri dish bottoms (60 mm diameter) were filled with distilled water, covered with parafilm, and placed in the bottom of each arena. Each arena contained two leaves from one plant of each *Rhododendron* species. There were five replicates of each arena. A total of 100 leaves of each species (taken from five plants of each species) was used in the experiment. Two beetles were placed in each arena. Prior to each test, the experimental beetles were fed for 24 h on leaves from the same *R. periclymenoides* plant and then starved for 8 h. This plant was not used in feeding tests.

The no choice feeding experiment included two tests (June 27-28). In this experiment, *R. atlanticum* was the experimental treatment and *R. periclymenoides* was the control. The feeding arenas were prepared as described for the paired choice tests except that there were only two leaves in each arena and both leaves came from the same plant. Five arenas each contained only leaves from *R. atlanticum* or *R. periclymenoides*.

In both experiments, the leaves were pressed and dried in a plant dryer at the end of each test. The dried leaves were taped to a sheet of paper and xerocopied. Leaf area and leaf area eaten were measured (mm^2) from the zeroxed leaves with a Zidas image analyzer. Each data point is the mean of two measurements. The results were expressed as percent leaf area eaten (%LAE) and as the number of leaves of each plant species sampled by the beetles. The data from the paired choice experiment were analyzed statistically by a Mann-Whitney U test and data from the no choice experiment by a chi-square test with Yates correction for continuity (Zar, 1984).

Results

In the paired preference test, beetles fed on 81% of the leaves of *R. periclymenoides* and 10% of the leaves of *R. atlanticum*. Mean %LAE was 3.53 ±4.04 (0.00-30.50) for *R. periclymenoides* and 0.05 ±0.18 (0.00-1.05) for *R. atlanticum*. The difference in mean %LAE between the two *Rhododendron* species is statistically significant ($U = 8904.00$; $P < 0.0001$.

In the no choice tests, beetles fed on all of the leaves of *R. periclymenoides* and one of the leaves of *R. atlanticum* ($N = 10$ for each species). Because of the small sample size, the data were analyzed with a chi-square test. The null hypothesis that the feeding data represented a 1:1 ratio was rejected at $P < 0.025$ ($X^2 = 6.400$; $df = 1$). In arenas in which only leaves of *R. periclymenoides* were present, the behavior of the beetles usually alternated between feeding and roaming freely about the container. In arenas in which only foliage of *R. atlanticum* was present, the beetles typically explored the leaves briefly and then moved to the top of the container and remained there for the duration of the experiment.

Discussion

The results of the paired choice experiment support my hypothesis that, in mixed populations of *Rhododendron periclymenoides* and *R. atlanticum*, *Pyrrhalta rufosanguinea* feeds preferentially on leaves of *R. periclymenoides*. The extremely low levels of feeding on *R. atlanticum* leaves in the no choice tests, as well as in the paired preference experiment, suggests that it is unlikely *P. rufosanguinea* would use this *Rhododendron* species as a host. In no choice tests, the difference in behavior of the beetles in the arenas of the two *Rhododendron* species, in addition to low feeding levels, suggests that they are repelled by *R. atlanticum* foliage. The chemical and morphological factors affecting host choice by *P. rufosanguinea* are under investigation. In other *Rhododendron* species, glandular scales on leaves have been associated with insect resistance (Doss, 1984). The leaves of *R. atlanticum* are glandular while those of *R. periclymenoides* are not; however, there are a number of other morphological and chemical differences between the two species.

Although results of both feeding experiments in combination with my previous field observations strengthen the case for monophagy, the feeding experiments should be repeated with larger sample sizes, longer starvation periods prior to each test, longer feeding trials and additional plant species. Saxena & Schoonhaven (1982) showed that prior experience can influence subsequent host choice by some herbivores. In this study, all beetles were fed on leaves from a single *R. periclymenoides* plant prior to feeding tests. In preliminary trials conducted before the experiments reported here, the beetles did not feed on *R. atlanticum* leaves. Therefore, I made no attempt to maintain them on *R. atlanticum* foliage before the feeding tests. Given the morphological and chemical similarities within *Rhododendron* alliances and differences between them, I suggest that *P. rufosanguinea* is an alliance-specific rather than a species-specific herbivore.

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Diabrotica cristata, a Seldom-Collected Leaf Beetle, Found on Buffalo Mountain, Floyd County, Virginia (Coleoptera: Chrysomelidae)

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The galerucine chrysomelid *Diabrotica cristata* (Harris) belongs to the same group of the genus as the well-known *D. barberi* Smith & Lawrence, the northern corn rootworm, and *D. virgifera virgifera* LeConte, the western corn rootworm. Unlike those pest species, however, *D. cristata* is seldom encountered by the general collector and poorly represented in insect collections. This univoltine leaf beetle is widespread east of the Rocky Mountains, particularly west of the Mississippi in relict Midwestern prairies (Wiesenborn & Krysan, 1980; Yaro & Krysan, 1986; Krysan & Smith, 1987). Its distribution along the eastern seaboard tends to be highly localized, and little is known about specific habitat preferences except for its occurrence in serpentine barrens of Maryland and Pennsylvania. Adults can be collected in serpentine barrens on inflorescences of grasses and forbs where they apparently feed on pollen. The larval host in eastern serpentine barrens was suggested to be little bluestem (*Schizachyrium scoparium* (Michx.) Nash) (Wheeler, 1988), but is more likely to be big bluestem (*Andropogon gerardii* Vitman), a perennial grass that serves as the larval host plant in Midwestern prairies (Yaro & Krysan, 1986; Krysan & Smith, 1987).

Herein *D. cristata* is recorded from Buffalo Mountain in southern Floyd County, Virginia, southeast of Willis. This monadnock, maximum elevation 1210 m, rises